Feburary 22, 1971

Dr. David Ward
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, W.C.2, England

Dear David:

Enclosed are 3 tubes of E. coli B restriction endonuclease in 50% glycerol. The enzyme proved stable in 50% glycerol at -50° for at least 10 days. This preparation is the G-200 Sephadex faction as described by Roulland-Dussoix and Boyer in BBA 195, 219, 1969; prior to glycerol addition it was in .01 M KPO_4, pH 7 - .005 M mercaptoethanol - .001 M EDTA. Tested against high specific activity, sonicated ^{32}P-T7 DNA (2×10^5 cpm/μg), it had no detectable exonuclease activity under the conditions of the B enzyme assay. To cleave SV40 DNA we use the following conditions:

SV40 DNA I - 20 γ/ml	6 λ
Mix -	4 λ
Enzyme (50% glycerol)	10 λ

36° x 30 minutes
Stop reaction by adding EDTA to .015 M

Mix:		
	SAM .06 M in .01 N H_2SO_4	10 λ
	Tris Cl pH 7.6 1 M	20 λ
	ATP .06 M	10 λ
	DTT .03 M	10 λ
	$MgCl_2$ 0.3 M	10 λ
	Water	40 λ

With the amount of enzyme given above 0.03 μg DNA was converted to a heat denaturable form in 5 minutes. The reaction slows down after about 15 minutes and generally I add more enzyme after 15 minutes. Recently I have found that the formation of form III proceeds at a decreasing rate for > 30 minutes and I suggest therefore that you incubate up to 1 hour.

To assay the enzyme we incubate for 5 minutes using radioactive DNA and add 5 volumes of 1 x SSC. This is heated at 100° for 2 minutes, quickly chilled in ice and passed through an S+S B-6 filter to count denatured DNA. One unit of enzyme is the amount that converts .01 μg SV40 DNA I to a denaturable form in 5 minutes.

I hope the enzyme works when it arrives and does what you need. Let me know if I can be of further help.

Best wishes,

Sincerely,

Daniel Nathans